45

65

62

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-continued
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The invention claimed is:

- 1. A CHO cell secreting a heterologous immunoglobulin obtainable with the following method:
 - a) providing a CHO cell, which is adapted to growth in suspension culture, adapted to growth in serum-free medium, and mycoplasma free,
 - b) providing a nucleic acid comprising
 - a prokaryotic origin of replication,
 - a first nucleic acid sequence conferring resistance to a prokaryotic selection agent,
 - a second nucleic acid sequence encoding the heavy 50 chain of said heterologous immunoglobulin, and a third nucleic acid sequence encoding the light chain of said heterologous immunoglobulin,
 - whereby a first transfection vector is provided which comprises said provided nucleic acid and an additional 55 fourth nucleic acid sequence conferring resistance to a first eukaryotic selection agent,
 - whereby a second transfection vector is provided which comprises said provided nucleic acid and an additional fourth nucleic acid sequence conferring resistance to a 60 second eukaryotic selection agent, whereby said second eukaryotic selection agent is different to said first eukaryotic selection agent,
 - b1) providing a nucleic acid comprising
 - a prokaryotic origin of replication,
 - a first nucleic acid sequence conferring resistance to a prokaryotic selection agent,

- a second nucleic acid sequence encoding the heavy chain of said heterologous immunoglobulin, and/or a third nucleic acid sequence encoding the light chain of said heterologous immunoglobulin,
- whereby a third transfection vector is provided which comprises said provided nucleic acid and an additional fourth nucleic acid sequence conferring resistance to a third eukaryotic selection agent, whereby said third eukaryotic selection agent is different to said first eukaryotic selection agent and is also different to said second eukaryotic selection agent,
- c) transfecting said CHO cell, wherein said transfecting comprises the following steps in the following order:
 - transfecting said CHO cell with said first transfection vector,
 - (ii) selecting a CHO cell transfected in (i) by selected growth in cultivation medium containing a first eukaryotic selection agent to which the first transfection vector confers resistance,
 - (iii) transfecting said selected CHO cell in (ii) with said second transfection vector,
 - (iv) selecting a CHO cell transfected in (iii) by selected growth in cultivation medium containing said first eukaryotic selection agent to which the first transfection vector confers resistance and said second eukaryotic selection agent to which the second transfection vector confers resistance,
 - (v) transfecting said CHO cell selected in (iv) with said third transfections vector,